CELL PROLIFERATION AS AN INDEX OF GROWTH IN CORALS; INCORPORATION OF ^{3}H-THYMIDINE[1)]

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With 4 Text-figures

Abstract

Preliminary experiments are described which are designed to quantify the rates of DNA synthesis in corals. Whole colonies or small sections of *Pocillopora damicornis* and other corals were incubated in up to 1 μCi ^{3}H-thymidine/ml sea water under field and laboratory conditions. The tissue was extracted with hot 2N NaOH and solubilized, and the ^{3}H-DNA activity was measured with a liquid scintillation spectrometer. Activity was expressed as CPM/mg dry or wet weight or CPM/calice. Specificity and site of the label was confirmed and studied with autoradiography and DNAse extraction. The label was primarily concentrated in the epidermis with maximum activity in the polyp columns and coenosarc. Labeling indices in the tentacles appeared to increase 5 to 7 days after ^{3}H-thymidine incorporation and this increase was probably due to migration of labeled cells from the polyp column. No ^{3}H-thymidine uptake occurred in zooxanthellae and very little was taken up by planulae.

Labeling between different calices of the coral species was uniform; however, decreasing rates of ^{3}H-thymidine incorporation were seen from apical polyps to basal branches in ramose forms. DNA synthesis in *P. damicornis* is markedly influenced by minor variations in incubation temperature. Optimum labeling occurred at an acclimation temperature of 31 °C with a rapid decline in rate on both sides. Indirect evidence demonstrates that the temperature optimum for the incorporation of ^{3}H-thymidine increases in parallel with the temperature of the habitat.

Introduction

Recent outbreaks of coral predators such as the Crown-of-Thorns starfish, *Acanthaster planci*, and the increased presence of pollutants in tropical waters have lead to greater interest in the survival and growth of corals and coral reefs. Growth in corals, primarily scleractinian colonial species, has been the subject of much experimentation and speculation. Measurements of linear growth and weight gain were recorded in the field by KAWAGUTI (1941), KNUTSON, BUDDEMEIER and SMITH (1972) and others, and have proved especially useful in assessing *in situ* characteristics of growth. Recently emphasis has been placed on growth determinations made under controlled laboratory conditions and the utilization of various substances which may be incorporated into the coral skeleton to quantify calcification.

Normally, weight or size increments are recorded in the laboratory over a 30 day

1) Contribution from the Marine Laboratory, University of Guam, Agana, Guam.

or longer period under varied treatments of temperature, nutrient supply or light (FRANZISKET, 1970; and JONES, University of Guam, personal communication). GOREAU (1959 and subsequent papers) developed a method using Calcium-45 to determine the growth rates of various parts of coral colonies. He applied this technique to a wide variety of species under varied environmental conditions. These methods, however, will not detect short-term fluctuations in biomass in response to proliferative changes in cellular elements, i.e., cell renewal or turnover. The rate of cell or tissue proliferation is known to be influenced by numerous pathological and experimental conditions, such as regenerative processes and changed environmental conditions (SCHULTZE, 1969). Therefore, measurement of cellular proliferation in corals may provide useful information on the influence of similar factors in coral growth and survival.

This paper describes the utilization of a tritiated thymidine label (^{3}H-TdR) to quantitate the rate of DNA synthesis in corals as an index of cellular proliferation or tissue growth. The studies have shown that (1) ^{3}H-TdR is incorporated quantitatively, (2) the rate of uptake can be modified over a short time span within non-lethal environmental tolerances, and (3) labeling is localized within specific cell types or areas.

Methods and Materials

Four species of scleractinian hermatypic corals were used in this study. They were *Pocillopora damicornis*, *Pavona decussata*, *Acropora formosa* and *Acropora aspera*. Most were gathered from reef flat or shallow water lagoons surrounding the island of Guam but a few were taken from reef terraces in deeper waters beyond the fringing reef. The colonies were collected entire or fragmented and were held in flowing sea water, for periods up to one month without feeding and under natural light.

Quantitation experiments were typically initiated by breaking the colonies or fragments into small sections, usually apical portions of branches. These sections were held for up to 24 hours under 500 foot-candles of fluorescent illumination in beakers or finger bowls containing 0.1 to 1 liter of unfiltered sea water (salinity 33 to 34‰). The water temperature and holding times were dependent on the design of the experiment and no attempt was made to orient the samples to any particular plane.

Labeling was begun by pulsing ^{3}H-TdR (SCHWARZ MANN, specific activity 3.0 Ci/mMole) directly into the container of coral sections or by transferring the specimens to a separate container of seawater doped with a specific amount of label. The amount of ^{3}H-TdR employed varied from 0.01 to 1 μCi per ml. At the end of an incubation period usually of 15 to 60 minutes, labeling was terminated by either flushing the samples with fresh sea water (for samples with low activity) or by transferring the corals to containers of unlabeled sea water. Further dilution of excess

unlabeled ^{3}H-TdR was accomplished by maintaining the corals in running sea water for 6 to 12 hours. The labeled, sea water-flushed samples were rinsed in distilled water (necessary for efficient solubilizing), drained for one minute, and placed in preweighed vials. The latter step was facilitated by incubating samples of uniformly small size and weight (0.1 to 1 g) or by subsampling larger blocks of labeled material to obtain the smaller sections. The sample vials were reweighed and 3 to 5 ml of 2N NaOH in distilled water was added (amount constant for each experiment). The samples were refluxed in a sandbath for 12 hours at 60° to 70° C to digest the tissue. They were cooled and 0.2 ml aliquots were added to scintillation vials containing 15 ml alkaline solubilizer (BECKMAN Bio-Solv, BBS-2). Three replicate counts of one to five minutes each were made at room temperature on a BECKMAN Beta-Mate II scintillation spectrometer.

The amount of coral tissue in each sample was indirectly quantitated by using wet weight of each coral specimen as a weighting factor for label activities. The number of calices and dry weights in labeled samples of *P. damicornis* were recorded in one experiment to ascertain their usefulness as alternate weighting factors. Evaluations of differences in ^{3}H-TdR uptake were compared with description of the size and colony of origin of each sample as well as physical characteristics of the sampling site.

To characterize the possible injurious effects of breaking the colonies apart prior to processing, one colony of *P. damicornis* was split immediately after removal from the field. One half was broken into 0.5 to 2.0 g tip sections, the other half was retained as an "uninjured" control. After holding all specimens for 48 hours in flowing sea water, the unbroken control was fragmented to nearly the same weight as the experimental group. All specimens were then labeled and processed.

A single rate-of-uptake experiment was conducted at ambient temperature (approximately 28 °C) with *P. damicornis*. Tip sections were incubated for 30 seconds to 4 hours in sea water containing 0.01 μCi ^{3}H-TdR/ml. Activity of the labeling solution decreased only slightly before and after the experiment. At times up to one hour after the initiation of incubation, specimens were transferred to a solution (10 μg/ml) of cold TdR for 15 minutes to inhibit further labeling. Otherwise, the labeled corals were processed as described previously.

A temperature rise of 3 to 8 °C over ambient sea water temperature was employed to modify the rate of ^{3}H-TdR uptake. Tip sections of *P. damicornis* and *Pavona decussata* were acclimated for 3 to 24 hours in thermostatically controlled water baths set at 28°, 31°, 34° and 36 °C. They were incubated at these temperatures in ^{3}H-TdR and the excess label was flushed ambient temperature.

Some qualitative data were gathered from field experiments in which unbroken tips of large colonies of *A. formosa* and *A. aspera* were labeled *in situ*. This was done by injecting 1 to 10 μCi of ^{3}H-TdR into sea water contained in small plastic bags held over the samples with rubber bands. Because the bags had a variable and undeter-

mined internal volume, the actual concentration of ^{3}H-TdR was unknown. Labeling was terminated after 15 minutes by removing the bags, and the sections were allowed to rinse for 24 hours before sampling. Sample processing was the same as noted for laboratory samples.

To determine the specificity and sites of ^{3}H-TdR uptake in *P. damicornis* labeled tip sections from the same colony were prepared for DNAse treatment. After fixation in 10% sea water formalin for 24 hours they were placed in a DNAse solution (1 mg/ml in pH 5.8 Tris-HCl buffer) and a new-DNAse control solution (pH 5.8 Tris-HCl buffer), incubated at 37°C for 15 hours and processed.

Microautoradiographs were prepared from labeled specimens which had been removed from a single colony and incubated for 60 minutes at ambient temperature in 0.1 μCi ^{3}H-TdR per ml sea water. The labeled corals were held in running sea water and sampled 1 hour to 7 days after labeling. Immediately upon sampling the corals were fixed in 10% sea water formalin for 24 hours. After fixation, they were decalcified in cold 10% HNO_3 in 70% ethyl alcohol, subsampled for scintillation counting, paraffin embedded and sectioned at 5 to 7 μ. The sections were stained with Harris hematoxylin and coated with undiluted Kodak NTB-2 liquid nuclear track emulsion. The coated slides were exposed for 1 or 4 weeks at 4°C, developed in Kodak Dektol developer (diluted 1:2) for 2 minutes, rinsed in distilled water and fixed in 30% sodium thiosulfate for 5 minutes. Most slides were counter-stained with Eosin-Y after photographic processing.

Results and Discussion

The Characteristics of ^{3}H-TDR Uptake in Corals

Tritiated thymidine is rapidly incorporated into the synthesizing DNA of the four coral species used in this study from concentrations less than or equal to 8.1×10^{-1} μg per ml sea water (3.3×10^{-9} M). DNAse demonstrates the specificity of the label in *P. damicornis* with a quantitative reduction of activity to a level close to background. Autoradiographs also convincingly show that activity is confined to nuclear labeling. The label is not retained in dead coral and formalin fixed samples incorporated no ^{3}H-TdR after "labeling." Exchange or loss of label into the surrounding water appears to occur at a slow rate in *P. damicornis*. Samples held for one week in aquaria had 70 to 100% of the original (1 hour post-labeling) activity; however, by 4 weeks activity had dropped to 10% of the original.

The rate of ^{3}H-TdR uptake in *P. damicornis* at ambient temperature was approximately linear up to two hours but became highly variable after three hours of incubation (Fig. 1). It is not possible with these data to separate cell transport from the biochemical requirements for ^{3}H-TdR. I assume that the two processes are combined, with transport, the limiting factor at lower thymidine concentrations.

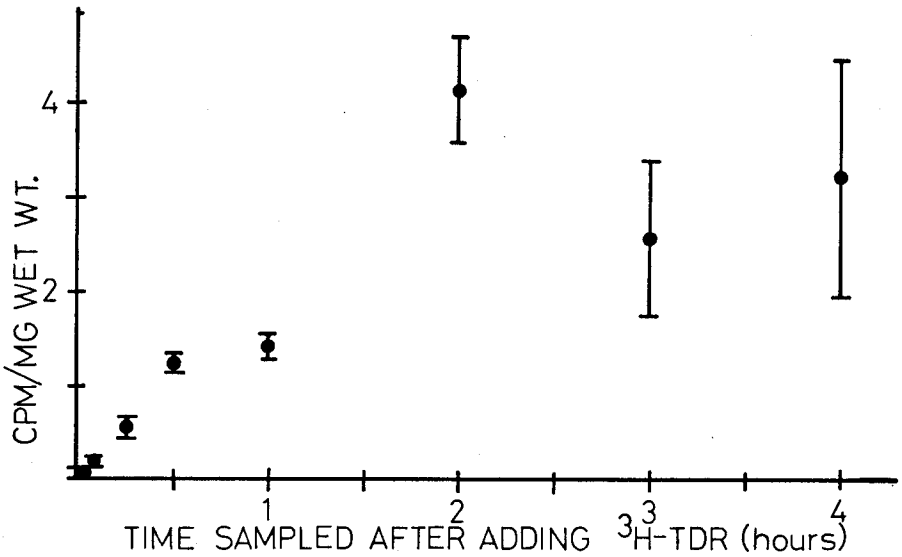

Fig. 1. Uptake of ^{3}H-TdR incorporated by *Pocillopora damicornis* as a function of time at 28 °C. Vertical bars indicate the range.

The work of STEPHENS (1960) and GOREAU, GOREAU and YONGE (1971) has shown that the uptake of small organic molecules such as glucose and amino acids in corals is concentration-dependent. However, uptake of these substances occurs at concentrations less than 3×10^{-9} M and is independent of light intensity.

The expression of ^{3}H-TdR uptake on the basis of total wet weight proved to be an acceptable method for the species used in my study. Correlation coefficients significant at $P<.01$ ($n=31$) were obtained for *P. damicornis* when relating wet weight to dry skeletal weight ($r=.991$), and the number of calices to dry skeletal weight ($r=.897$) or to wet weight ($r=.907$). Similar values were derived by CLAUSEN (1971) for *P. damicornis* from Hawaii. The repeatibility of these parameters is dependent on a number of factors. Wet and skeletal weights are subject to surface, volume, density, and mineralization (dry weight/protein N) variations between and within colonies and can only be used when the colony can be sampled in discrete units. Counts of calices are reliable only when polyps are small, evenly and closely spaced; however, they could be applied to massive colonies.

Labeling site gradients—distal to proximal changes in activity on colony branches —are not significant in *P. damicornis* and untested for *Pavona decussata.* The uptake of label in *Acropora formosa* and *A. aspera* per unit wet weight is greatest at and adjacent to the apical polyp and decreases proximally. The activity of the apical polyp is 2 to 10 times as great as the activity on the column of these ramose species. This is analogous to the distribution of calcification rates measured in similar species of ramose corals such as *Acropora cervicornis* and *Porites furcata* by GOREAU (1961).

Parasite-induced or "tumor-like" galls found on *A. formosa* stimulate DNA synthesis and lead to rates equivalent to apical polyp activities. In addition, sections which include galls of various sizes usually have higher activities per unit weight than do equivalent normal sections. Average counts per minute per gram wet weight in a series of six observations were 8500±2322 SE for normal sections and 12,900±1300 SE for abnormal sections.

Comparisons of the ^{3}H-TdR uptake of *A. formosa* and *A. aspera* reveal much higher activities for the latter species. The activity of *A. aspera* is two to three times as high as the highest activity measured in *A. formosa.* While they are generally similar in appearance, *A. aspera* has thinner, more numerous branches and a lighter more porous skeleton than *A. formosa.* This implies faster growth and may account for the higher DNA synthesis activity seen in *A. aspera.*

Of the four species studied, *A. formosa* and *A. aspera* proved most difficult to use in extended laboratory studies. Handling these species invariably resulted in injury and rapid necrosis of tissues adjacent to the injury. Neither remained viable beyond a few weeks when held in aquaria. However, field studies with *A. formosa* and *A. aspera* did not have this limitation and were hampered only by poor standardization of the label dose. They pointed out the usefulness of the method to explore *in situ* ^{3}H-TdR labeling without the interference of laboratory-induced artifacts.

P. damicornis exhibits a delayed injury response to handling similar to mammalian systems where cell proliferation stimulated by injury reaches peak levels 24 to 48 hours after injury (MCMINN, 1969). In samples labeled 48 hours after injury, average counts per minute per gram wet weight of the injured colony were 1287±114 SE (n=3). The "uninjured control colony" (the undamaged half colony) averaged 989±43 SE (n=3). BRAVERMAN (1969) recorded peak ^{3}H-TdR labeling indices from autoradiographs six hours after injury in the hydroid, *Podocoryne carnea.* At 24 hours post-injury, his label activities had decreased to near normal levels.

Localization of DNA Synthesis in Pocillopora damicornis

Autoradiographs clearly demonstrate the specificity of labeling within particular cell types although the duration and cytologic resolution of the experiments were not sufficient to allow complete assessment of cellular differentiation. The distribution and relative intensity of ^{3}H-TdR incorporation is shown in Fig. 2, for a representative section combining data from samples taken one hour to seven days after labeling. The activity was generally concentrated in the column and coenosarc of the polyp. In longitudinal section proximal polyps had the same relative labeling indices as polyps up to 2 cm distal to them. The epidermis always had higher labeling indices when compared to endoderm with the activity apparently dominated by interstitial cells and epithelial-muscular cells. Zooxanthellae incorporated no ^{3}H-TdR and very little was taken up by planulae even when they were adjacent to highly labeled epidermal sites. A large number of unlabeled mitotic figures were seen in planulae

Table 1 Average labeling indices (% labeled nuclei) and ranges in epidermal nuclei of *Pocillopora damicornis* labeled for 30 minutes with 0.1 μCi/ml and sampled after various time intervals.

Time sampled	Labeling indices in nuclei	
	Base of column wall	Tips of tentacles
1 hour	9 (8 – 10)	1 (0 – 1)
1 day	13 (10 – 16)	1 (0 – 2)
5 days	10 (6 – 14)	3 (2 – 4)
7 days	8 (6 – 9)	4 (3 – 6)

epidermis and smaller numbers were observed in the polyp columns.

Table 1 presents the changes in labeling indices in the column and tentacles of polyps with respect to time after labeling. These data suggest that a large fraction of the nuclei labeled in the polyp column (Fig. 2) contribute labeled elements to the tentacles. BRAVERMAN (1969) observed a similar pattern of ^{3}H-TdR incorporation in the colonial marine hydroid, *Podocoryne carnea*. One hour after labeling, DNA synthesis is primarily concentrated in the hypostome region of the gastrodermis and in the mid-body of the epidermis. He felt that these areas constitute zones of proliferation supplying cells to the holocrine glands of the hypostome ridges and to the tentacle gastroderm. Essentially the same conclusions were reached by CAMPBELL (1965) after an autoradiographic study of ^{3}H-TdR labeled *Hydra*. He and BRAVERMAN (1969) believe that the proliferative zone is rather broad and occupies the whole mid-column region. This concept of diffuse proliferation as defined by DNA synthesis would certainly also apply to *P. damicornis*.

Little variation was noted in the activity of the endoderm (Fig. 2). The decreased

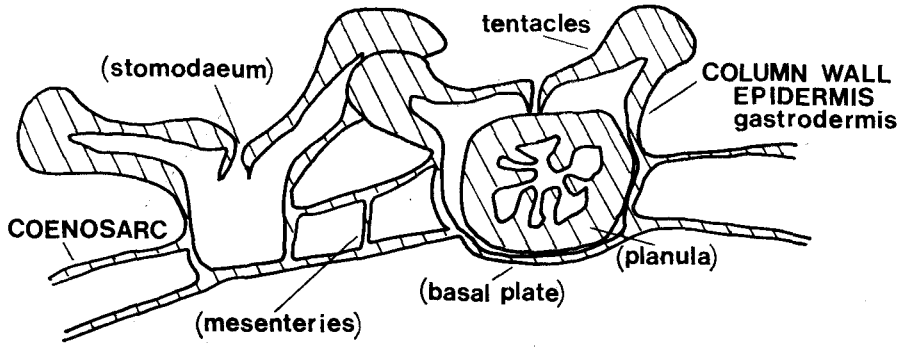

Fig. 2. Diagrammatic vertical section of the polyps of *Pocillopora damicornis*. The relative percentage of ^{3}H-TdR labeling in nuclei is indicated for the respective areas or structures by character format. Upper case=high index, always labeled. Lower case=low index, always labeled. Lower case in parenthesis=low index, labeled only in a few samples. Other structures, exhibiting no labeling are unmarked.

uptake of label by this tissue cannot be definitely ascribed to reduced cell proliferation because the stomodaea did not always open during labeling, although the polyps and tentacles remained extended during most of the incubation period. It is possible that the amount of label reaching the endoderm was limited only to that portion which could diffuse through the epidermis. Nevertheless, the pattern of ^{3}H-TdR labeling does not differ significantly from observations of amino acid incorporation by GOREAU, GOREAU & YONGE (1971). Using similar incubation times and concentrations, they found ^{3}H-DL-leucine activity initially fixed in tall columnar cells of the epidermis, with much less in the gastrodermis, very little in the mesoglea and none in the zooxanthellae. After 24 hours the label becomes more uniformly spread throughout the tissue, but zooxanthellae remain unlabeled.

Temperature and DNA Synthesis

DNA synthesis in *P. damicornis* is markedly influenced by minor variations in incubation temperature, and the data are in general agreement with the observations of temperature-induced variations in the synthesis of macromolecules in mammalian and bacterial systems (FEINENDEGEN, 1967). The typical uptake curve for *P. damicornis* is shown in Fig. 3. These data were taken from specimens acclimated for three hours

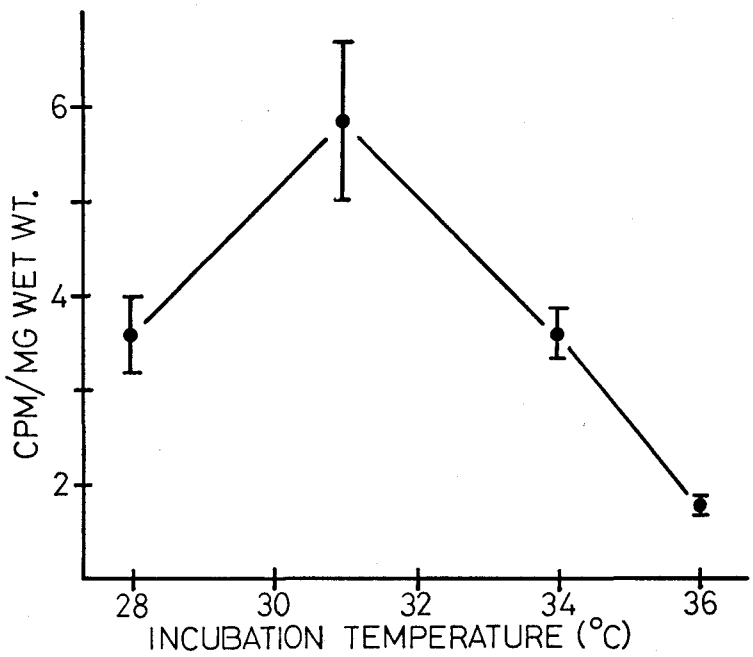

Fig. 3. The effect of temperature on the rate of incorporation of ^{3}H-TdR by *Pocillopora damicornis*. Corals were acclimated for three hours and incubated at acclimation temperatures in 0.5 μCi ^{3}H-TdR per ml sea water for one hour. Vertical bars indicate $\pm$ one standard error (n=9).

at incubation temperatures shown and then incubated for 60 minutes in 0.05 μCi ^{3}H-TdR per ml sea water. Count-per-minute variations were significant in single classification analyses of variance at all water temperatures ($P<0.01$), at 28°, 31° and 34°C only ($P<0.05$) and at 28° and 31° only ($P<0.05$). The experiments were repeated with similar results, with one exception, for nine colonies, including one taken from 15 meters. The highest incubation temperature (36°C) was lethal to *P. damicornis* if acclimation exceeded 12 hours. *Pavona* did not respond to the temperature regime and failed to show any significant changes in activity through the 8°C (28° to 36°C) test span after acclimation for 24 hours.

A difference was noted in temperature responses between colonies of *P. damicornis*. Figs. 4a to 4c show the labeling activities recorded in a single experiment employing replicates of four colonies with three observations per colony. Three colonies (Nos. 2–4) were taken from the same tide pool on the reef flat and processed eight hours after sampling. One colony (No. 1) was held for 30 days in running sea water prior to processing. The colonies were acclimated for seven hours and then incubated for one hour with 0.1 μCi ^{3}H-TdR per ml. Three of the colonies present an uptake picture which was equivalent to Fig. 3, but one colony (No. 2) has decreased activities at 31° and 34°C. The response curves are essentially the same for both wet and dry weights (Fig. 4a & 4b) and for calice counts (Fig. 4c); however, the one colony (No. 1) held in running sea water for 30 days has much lower overall activities than the other replicates. This phenomenon was seen in other colonies held for extended periods in aquaria supplied with flowing sea water and indicates a physiological disturbance of unknown origin. The incubation medium was monitored at the start and at the conclusion of the experiment. The differences in activities were 2898 CPM at 28°C, 1722 CPM at 34°C, and 793 CPM at 36°C. Possibly, as the incubation temperature increased, the rate of incorporation decreased, or the degradation of thymidine increased, or there was a combination of these events.

There is not a substantial difference between temperature-dependent uptake of ^{3}H-TdR or ^{45}Ca. Optimal ^{45}Ca incorporation in *P. damicornis* (CLAUSEN, 1971) and *Fungia scutaria* (YAMAZATO, 1970) in Hawaii was at 25°C with a rapid decline in rate on both sides. A similar observation was made by HEATFIELD (1970) for regenerating spines of the urchin *Strongylocentrotus purpuratus* from California which showed maximum uptake at 20°C for an even lower series of incubation temperatures. If the kinetics of calcification and DNA synthesis can be equated it would appear then that *P. damicornis* in Hawaii (average ambient water temperatures 22° to 27°C) are acclimated to water temperatures 5° to 6°C below the optimum acclimation temperature of the Guam *P. damicornis* (average ambient water temperatures 26° to 29°C). Information on lethal temperatures for *P. damicornis* in Hawaii (JOKIEL, University of Hawaii, personal communication) and in Guam (JONES, University of Guam, personal communication) support this view, with long-term survival limited at temperatures equivalent to the maximum limits noted by CLAUSEN (1971) and myself.

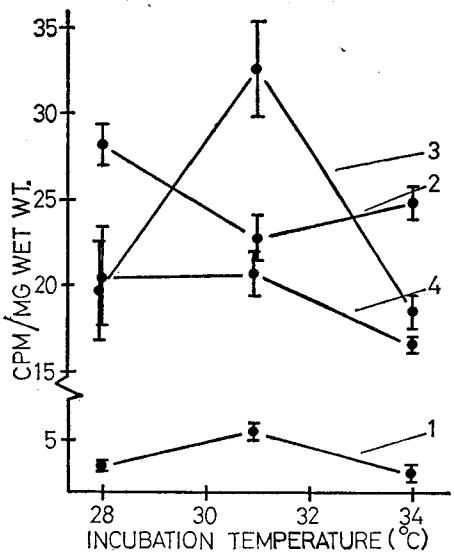

Fig. 4a. The effect of temperature on the rate of incorporation of ^{3}H-TdR by *Pocillopora damicornis*. Samples were acclimated for seven hours and incubated at acclimation temperature in 0.1 μCi ^{3}H-TdR per ml sea water for one hour. Vertical bars indicate $\pm$one standard error (n=3). Numbers indicate individual colony designations noted in the text. Activity is expressed in mg units of total wet weight.

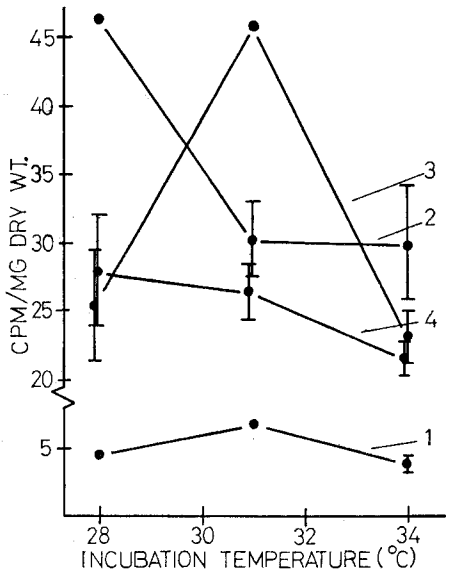

Fig. 4b. Same as Fig. 4a but activity is expressed in mg units of dry or skeletal weight.

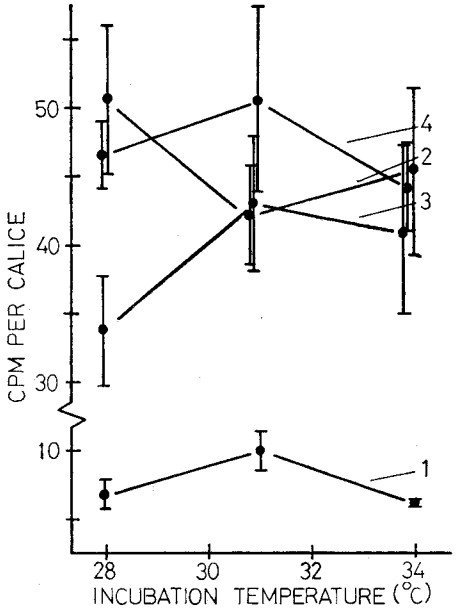

Fig. 4c. Same as Fig. 4a but activity is expressed in calice units.

Conclusions

Coral growth is the combined result of a large number of factors which can in part be expressed with parameters of weight and size gains, oxygen production or utilization, or the uptake of certain radioisotopes. The measurement of DNA synthesis in corals with the incorporation of labeled precursors is obviously dependent on these factors plus a large number of other intrinsic and extrinsic components. However, it is likely that most of the underlying assumptions concerning the uptake of ^{3}H-TdR into tissues apply to corals.

While DNA synthesis in corals was quantified in different species in units based on weight and number of calices, it is difficult to apply weighted activities to comparisons between species. An approach to this problem would be to determine total DNA for each ^{3}H-TdR active sample. If the surface, or epithelial, labeling picture noted in autoradiographs is confirmed, total DNA estimates would have to be corrected for reproductive state of the colony and numbers of zooxanthellae.

The uptake of ^{3}H-TdR by coral proliferocytes is probably not an absolute measure of growth. Evidence is given for the existence of cell renewal systems in the tentacles and gastrodermis with cellular elements originating in growth zones. I assume that the cells in these systems originate from interstitial cells, which in *Hydra* at least are totipotent and account for the replacement of cnidoblasts, neurons and gland cells (LENTZ, 1966). Therefore, only a portion of the proliferocyte compartment in the coral polyp will be involved in tissue growth. The absence of labeling in zooxanthellae appears to verify the vegetative nature of these cells, although it was stated by YONGE (1963) that they do undergo fission. It is possible that thymidine "salvage" mechanisms may be dominated by *de novo* synthesis in zooxanthellae, thereby limiting uptake from an external source.

P. damicornis is a sensitive indicator of fluctuations in incubation temperature, both when related to DNA synthesis and to calcification (CLAUSEN, 1971). The acclimation times of most experiments (3 to 24 hours) were probably within normal ranges in duration and, at lower incubation temperature, amplitude of natural temperature fluctuations. Indirect evidence demonstrates that the temperature optimum for the incorporation of ^{3}H-TdR and ^{45}Ca increases in parallel with the temperature of the habitat. This evidence is supported by some recent investigations by ZEIKUS and BROCK (1972), who had access to a relatively stable fresh water thermal gradient. Following ^{45}C-glucose incorporation in relation to temperature in benthic bacterial populations they concluded that the bacteria were near-optimally adapted to the temperature of their habitat and that despite wide differences in temperature the rate of bacterial activity was the same. A similar acclimation pattern probably exists for many coral species distributed widely across broad temperature gradients.

There are a great many questions which remain to be answered regarding cellular

aspects of coral growth. Unknown are the effects of light-dark regimes and diurnal, tidal and seasonal variations on proliferative activity. Further experimentation should also be aimed at determining the relationship of acclimation time to proliferative activity, and in quantitating recovery after environmental insults. The quantification of these data will characterize an important aspect of coral physiology under normal and stress conditions.

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DISCUSSION

ROSS: Can you ascribe the low uptake in animals retained in the laboratory for 30 days to

changes in certain cells or at certain locations in the colony?

CHENEY: Not yet; however, this problem is now under study at our laboratory with comprehensive autoradiographic and quantitative analyses.

BRAVERMAN: Why do you use such small labeling doses of thymidine?

CHENEY: High labeling activities were obtained with doses well above the known lower concentration limits of corals.

YAMAZATO: Working with corals I have found it fairly difficult to keep their mouths open while conducting some experiments. I am wondering whether your animals kept their mouths open while you were labeling them with ^{3}H-thymidine?

CHENEY: I am not sure, but they were relaxed and tentacles were extended throughout most of the incubation period.